54. A method according to Claim 25 wherein the ratio or ratios calculated in step (c) when compared to said mean or means of ratios indicates that the abnormally low level of said wild-type protein expressed by one of the subject genes in said sample is about 50% of the level of said wild-type protein in comparable samples from organisms unaffected by said disease or said disease susceptibility trait.

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REMARKS

To assist in the examination of this application and as required by 37 CFR 1.121, enclosed herewith as Appendix 1 is a marked-up version of the amendments to the claims. The modifications are indicated by underlining and in bold type for additions, and by strikeouts for deletions. Also enclosed as Appendix 2 is a clean set of all the claims now pending in accordance with 35 CFR 1.121(c)(3).

The claims have been amended to point out with more particularity and clarity the subject matter which is regarded by the Applicant as his invention. Applicant respectfully submits that no new matter has been entered by the above amendments to the claims.

All the claims have been amended to concern methods of detecting a disease or disease susceptibility trait associated with the germline mutation. As the Background of the Invention states at page 1, lines 18-27:

> [I]t is valuable to know whether a person has a germline or an acquired mutation associated with a disease or susceptibility to a disease.
>
> For example, pre-operatively identifying a colon cancer patient who carries a germline mutation in a mismatch repair gene associated with hereditary non-polyposis colon cancer

> (HNPCC) guides a surgeon in deciding whether or not to perform a total colectomy or a partial colectomy. If the patient has a germline mutation, then the chance for a second primary colon cancer is extremely high (perhaps 70%). In that case, a total colectomy is usually recommended as the initial surgical treatment.

[Emphasis added.]

Then, the instant application at page 2, lines 7-14 states:

> In addition, positive diagnoses of germline mutations in cancer patients will facilitate testing for detection of germline mutation carriers in their family members. Such testing will lead to more effective cancer prevention strategies and to better clinical management for cancers that are detected in family members. Thus, the information provided by this invention will also be useful to the physician in recommending the screening of other members of the patient's family since the finding of a germline mutation puts those individuals at high risk for colon cancer and other cancers.

[Emphasis added.] The Summary of the Invention at page 5, line 32 to page 6, line 2 states: "The immunoassay methods of this invention are ... premised on the assumption that germline mutations in two different genes of one individual are very rare." [Emphasis added.]

The claims have also been amended to indicate that the biological samples tested are normal samples. The application at page 12, lines 7-10 reads that "[f]urther preferred" biological samples are:

> normal cell samples, normal cell extracts, lysates of normal cells, and supernatants of normal cell lysates. Particularly preferred are samples of peripheral blood lymphocytes (PBLs), lysates of PBLs, supernatants from lysates of PBLs, and extracts of PBLs.

[Emphasis added.] Claims 9, 11, 33 and 35 have also been amended to reflect that the samples contain normal, non-neoplastic cells.

The claims have further been amended to claim methods of determining whether or not the calculated ratio of wild-type protein expressed by a subject gene reflects not the absence of that wild-type protein but an abnormally low level of wild-type protein, signifying a mutation in only one of the alleles of the subject gene.

New Claims 49-54 point out that an abnormally low level of wild-type protein found when one allele of a subject gene contains a germline mutation is about 50%, with a range of deviation from 50% being ± 20%, ± 15%, or ± 10%, of the amount of said wild-type protein found in comparable samples from organisms of the same taxonomic classification that are unaffected by the disease or disease susceptibility trait associated with said germline mutation. New Claims 49-54 are supported in the application at least at page 5, line 24 to page 6, line 20; at page 9, line 22 to page 10, line 2; at page 11, lines 19-21; at page 19, line 27 to page 20, line 3; at page 45, lines 24-29; at page 49, lines 1-25; and at page 50, lines 1-5. Specifically the ranges of Claims 49-54 can be found at page 9, line 32 to page 10, line 2.

The Summary of the Invention at page 5, line 24 to page 6, line 20 reads in pertinent part:

> The immunoassay methods of this invention are based on the theory that normal individuals, that is, those without a mutation in a subject gene, will have 100% expression of that subject wild-type gene product and also 100% expression of a wild-type reference gene product. In contrast, an individual with a mutation in an allele of that same gene will in theory have only 50% expression of the subject wild-type gene product....
>
> . . . The immunoassay methods of this invention are also in this aspect premised on the assumption that germline mutations in two different genes of one individual are very rare.

> Representative immunoassays of this invention are those to detect susceptibility to HNPCC [hereditary non-polyposis colon cancer] and FAP [familial adenomatous polyposis] in humans. In the case of screening for the HNPCC susceptibility trait, the amounts of MLH1 and MSH2 wild-type proteins (the expression products of the two major MMR [mismatch repair] genes) are measured from a sample from an individual, e.g. from freshly prepared lymphocytes. Almost all individuals with a germline MMR mutation will have 100% of one of those two full-length MMR proteins but only 50% of the other full-length protein.
>
> . . . For example, in the case of MMR mutations, the ratio of the amount of MSH2 protein to the amount of MLH1 protein can be used. It is then determined whether the numerical value of the ratio falls clearly in a normal range or clearly in a range predicted for 50% loss of expression of the subject gene product, e.g., 50% loss of expression of either MSH2 or MLH1 protein when screening for HNPCC.

[Emphasis added.]

The application states at page 11, lines 19-21: "When the assay results indicate that the subject gene has a mutation in one allele, the abnormally low amount of wild-type protein is generally about 50% of the control amount, with the above-noted variability [i.e., ± 20%, preferably ± 15%, more preferably ± 10%] being operative."

Further "[t]he assays of this invention are based on the assumption that gene expression directly relates to gene dosage, that is, the presence of two wild-type alleles will result in the expression of twice the amount of full-length wild-type protein than would occur if only one wild-type allele were present." [Application, page 2, lines 24-27.]

Example 1 at pages 45-50 of the instant application:

> supports the theory underlying the immunoassays of this invention in that the lymphoblastoid cells from the FAP patients were shown to have **about 50% less** (50.1% ± 5.1%) immunoprecipitable full-length APC protein in comparison to

controls lacking germline APC mutations. The results correlate with the heterozygous APC genotypic status in FAP cells.

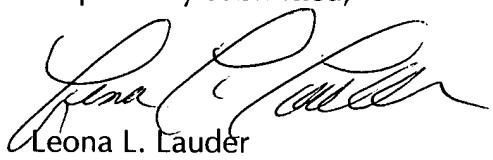
[Application, page 45, lines 24-29; emphasis in bold added.]

Applicant respectfully concludes that no new matter has been entered by the amendments to Claims1-48 including the cancellation of Claims 7, 8, 29 and 30, or by the addition of new Claims 49-54.

CONCLUSION

Applicant respectfully concludes that the claims as amended are in condition for allowance, and earnestly request that the claims be promptly allowed. If for any reason the Examiner feels that a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to telephone the undersigned Attorney for Applicant at (415) 981-2034.

Respectfully submitted,



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Attorney for Applicants
Registration No. 30,863

Dated: April 26, 2001

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APPENDIX 1

Claim 1 on page 60, line 1-21 has been amended as follows

1. (Amended) A method of detecting a disease or a disease susceptibility trait in an organism, wherein said disease or said disease susceptibility trait is associated with a **germline** mutation ~~or mutations~~ in a subject gene, comprising:

(a) isolating a **normal** biological sample from said organism;

(b) immunologically quantitating the amount of wild-type protein expressed by said subject gene in said sample, and the amount of a reference protein expressed by a second gene in said sample;

(c) calculating the ratio of the amount of the wild-type protein expressed by said subject gene in said sample to the amount of the reference protein expressed by said second gene in said sample;

(d) determining ~~whether or not any wild-type protein expressed by said subject gene is present in said sample, or~~ whether or not said calculated ratio reflects an abnormally low level of said wild-type protein expressed by said subject gene in said sample; and

(e) concluding ~~that if no wild-type protein is present in said sample, that said subject gene contains a mutation in each of its alleles, or,~~ that if the ratio calculated in step (c) indicates that there is an abnormally low level of wild-type protein in said sample, that said subject gene contains a **germline** mutation in one of its alleles, and that ~~if either is the case, that~~ the subject organism is affected by the disease or the disease susceptibility trait associated with said **germline** mutation ~~or mutations~~.

Claim 9 on page 61, lines 12-15 has been amended as follows

9. (Amended) The method according to Claim 1 wherein said biological sample is selected from the group consisting of body fluids, tissue specimens, tissue extracts, **normal** cells, ~~cell~~ lysates **of normal cells**, **normal** cell extracts, **and** supernatants from ~~normal cell~~ lysates **of normal cells** ~~, supernatants from preneoplastic cell lysates, and supernatants from neoplastic cell lysates~~.

Claims 11 and 12 on page 61, lines 23-30 have been amended as follows

11. (Amended) The method according to Claim 9 wherein the cells are peripheral blood lymphocytes; the cell lysates are lysates of peripheral blood lymphocytes; the cell extracts are from peripheral blood lymphocytes; and the ~~lysates of normal, preneoplastic or neoplastic~~ supernatants are from **lysates of** peripheral blood lymphocytes.

12. (Amended) The method of Claim 1 wherein said biological sample is selected from the group consisting of ~~tissue samples, tissue extracts,~~ normal cells, lysates of normal cells, and supernatants from lysates of normal cells.

Claims 14 and 15 on page 62, line 4-11 has been amended as follows

14. (Amended) The method of Claim 1 wherein said **germline** mutation is ~~or said mutations are~~ selected from the group consisting of truncating-causing mutations and mutations that cause allelic loss.

15. (Amended) The method of Claim 14 wherein said mutation is ~~or said mutations are~~ selected from the group consisting of nonsense mutations, frameshift mutations, promoter mutations, enhancer mutations, splice site mutations, null mutations, and poly-A tail mutations.

Claim 24, page 63, line 21 to page 64, line 11 has been amended as follows

24. (Amended) A method of detecting a disease or a disease susceptibility trait in an organism, wherein said disease or said disease susceptibility trait is associated with a **germline** mutation ~~or mutations~~ in one of two or more subject genes, comprising:

(a) isolating a **normal** biological sample from said organism;

(b) immunologically quantitating the amount of wild-type protein in said sample, that is expressed by each of the subject genes;

(c) calculating the ratio of the amount of the wild-type protein expressed by one of said subject genes in said sample, to the amount of wild-type protein expressed by the other subject gene in said sample, or to each of the amounts of wild-type protein expressed by each of the other subject genes in said sample;

(d) determining ~~whether a wild-type protein expressed by either of the subject genes, or by any of the subject genes is absent from said sample, or~~ whether the ratio or ratios calculated in step (c) reflects or reflect an abnormally low level of a wild-type protein expressed by either of the subject genes, or by any of the subject genes in said sample; and

(e) concluding that ~~if a wild-type protein known to be normally expressed by one of the subject genes is not present in said sample, that that subject gene contains a~~

~~mutation in each of its alleles, or that~~ if the ratio or ratios calculated in step (c) indicates or indicate that there is an abnormally low level of a wild-type protein expressed by one of the subject genes in said sample, ~~concluding~~ that that subject gene contains a **germline** mutation in one of its alleles~~;~~ , and~~, in either case, determining~~ that the subject organism is affected by the disease or the disease susceptibility trait associated with said **germline** mutation ~~or mutations~~.

Claims 31 and 33, page 65, lines 1-13 have been amended as follows

31. (Amended) The method of Claim 24 wherein said mutation is ~~or wherein said mutations are~~ selected from the group consisting of truncating-causing mutations and mutations that cause allelic loss.

32. (Amended) The method of Claim 31 wherein said mutation is ~~or said mutations are~~ selected from the group consisting of nonsense mutations, frameshift mutations, promoter mutations, enhancer mutations, splice site mutations, null mutations, and poly-A tail mutations.

33. (Amended) The method of Claim 24 wherein said biological sample is selected from the group consisting of body fluids, tissue specimens, tissue extracts, **normal** cells, ~~cell~~ lysates **of normal cells**, **normal** cell extracts, **and** supernatants from ~~normal cell~~ lysates **of normal cells**~~, supernatants from preneoplastic cell lysates, and supernatants from neoplastic cell lysates~~.

Claims 35 and 36, page 65, lines 21-28 have been amended as follows

35. (Amended) The method of Claim 33 wherein the cells are peripheral blood lymphocytes; the cell lysates are lysates of peripheral blood lymphocytes; the cell extracts are from peripheral blood lymphocytes; and the **supernatants are from** lysates of ~~normal, preneoplastic or neoplastic supernatants are from~~ peripheral blood lymphocytes.

36. (Amended) The method of Claim 24 wherein said biological sample is selected from the group consisting of ~~tissue samples, tissue extracts,~~ normal cells, lysates of normal cells, and supernatants from lysates of normal cells.

Claim 45, page 67, lines 6-22 has been amended as follows

45. (Amended) A method of detecting a disease or a disease susceptibility trait in an organism, wherein said disease or said disease susceptibility trait is associated with a **germline** mutation ~~or mutations~~ in a subject gene, comprising:

(a) isolating a sample of normal cells from said organism;

(b) immunologically quantitating the amount of wild-type protein expressed by the subject gene in said sample;

(c) determining whether ~~any wild-type protein is present in said sample, and if so, whether~~ the amount of wild-type protein present in said sample is abnormally low in comparison to the amount of wild-type protein expressed by the subject gene in a control sample; and

(d) ~~if said wild-type protein is not present in said sample, concluding that the subject gene has a mutation in each of its alleles; or~~ if the amount of said wild-type protein

in said sample is determined to be abnormally low in comparison to the amount of wild-type protein in the control sample, concluding that the subject gene has a mutation in one allele~~,~~ and ~~in either case,~~ correlating the conclusion with the subject organism having the disease or the disease susceptibility trait associated with said **germline** mutation ~~or said mutations~~.